FUNGITOXICITY OF LIGNIN MONOMERS, MODEL SUBSTANCES, AND DECOMPOSITION PRODUCTS

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SUMMARY

Various humus preparations and lipids extracted from soils and ethanol extracts of natural soils were nontoxic or stimulatory to conidial germination and germ tube growth of *Glomerella cingulata*, *Penicillium frequentans*, and *Fusarium oxysporum* f. *lycopersici*, when tested in agar media. The lignin-like fraction extracted with dioxane or ethanol from washed and defatted soils and wheat straw and ethanol extracts of soil which had been amended with spruce native lignin inhibited spore germination and germ tube growth in variable degree. Spruce native lignin was nonfungitoxic, but various compounds related to lignin building units inhibited spore germination and germ tube growth in agar at concentrations of $10^{-2}M - 10^{-3}M$. The pH of the medium and autoclaving increased or decreased fungitoxicity of these compounds, but not in a consistent pattern. Fungistasis by some of the compounds was annulled by addition of 0.5% peptone. The possibility that lignin decomposition products may play a role in the beneficial effects of some mature crop residues on soil-borne diseases and in the widespread soil fungistasis is suggested by these results.

During an investigation of the source and nature of natural soil fungistasis (13), it was found that the fraction containing lignin-like material extracted from washed and defatted soil was fungitoxic (12). Inasmuch as lignin comprises up to ⅓ of the dry matter of plant material (1, 21) and is resistant to microbial attack, it accumulates in soil; whereas other constituents disappear (4, 20). Thus, soil organic matter is comprised of a large proportion of lignicolous material, a part of which may be complexed with protein to form humus (23). Although resistant to microbial attack, lignin is decomposed slowly by soil microorganisms (1, 5) under aerobic conditions. The metabolism of spruce native lignin (6) and some lignin monomers and model substances (8, 9) by fungus mycelium has been studied, but possible inhibitory effects of these compounds on the germination of fungus spores and growth of germ tubes apparently have not been studied. In view of the natural fungistasis of soils and the persistence of relatively large amounts of lignin in soil, the effect of lignin, lignin decomposition products, and related compounds on fungus spore germination and growth of germ tubes was investigated.

In a previous brief report (12) of some of this work. fungitoxicity of spruce native lignin and inhibitive effects of lignin-like materials extracted from soil and wheat straw at very low concentrations (60-80μg/ml) were reported. These results are now considered incorrect and were probably due to the use of ethyl ether and possibly of dioxane in the preparation of these materials for assay. Both of these solvents accumulate peroxides which oxidize lignin (1) to fungitoxic compounds, as shown in the present work.

MATERIALS AND METHODS.—Conidia of *Glomerella cingulata* (Ston.) Spauld. & Schrenk. *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyd. & Hans., and *Penicillium frequentans* Westling were obtained from 4-7-day-old cultures growing on potato-dextrose agar (infusion from 200 g potatoes, dextrose 20 g, agar 20 g in 1 liter) at 24°C.

Impure lignin-like substances were obtained from mature wheat straw, muck soil, and Conover loam soil by a method similar to that of Brauns (1) for extracting lignin from ground wood. This involved washing 100 g of finely powdered material with 10 volumes of distilled water at 21°. A Waring Blendor was used to pulverize plant material. The residual material was air-dried and extracted with petroleum ether (B. P. 30-60°C) in a Soxhlet apparatus for 6 hours to remove lipids. The defatted product was air-dried, then extracted with 200 ml of 95% dioxane or ethanol. Extraction was done 4 times, each for a period of 24 hours, on a shaking machine. The solvent was evaporated under vacuum, the products were dissolved in 10-20 ml quantities of 95% ethanol, and stored in this solvent at 24°C. The material was not purified further.

Fungitoxicity of crude extracts and of defined chemical compounds, all dissolved in 95% ethanol, were assayed by mixing them with melted, warm 2% water agar (Bacto) and pouring them into petri dishes. An ethanol solution of spruce native lignin was sometimes poured into petri dishes and the ethanol evaporated under vacuum. Water agar was poured over the residue. Plates were incubated for 48 hours at 24°C to permit diffusion. Spores of the 3 test fungi were streaked on triplicate agar plates containing the above extracts or chemicals. After 24 hours of incubation at 24°C, percentage of germination and length of germ tubes of approximately 300 spores were recorded. Maximum concentration of ethanol used was 3% which did not interfere with germination of spores or germ tube growth.

Infrared absorption spectra of spruce native lignin and dioxane extracted lignin-like material from muck and wheat straw were determined in mineral oil (Nujol) by Dr. S. Venkob Rao at the H. M. Randall

TABLE 1. Inhibitive effects of lignin-like substances from soils and wheat straw assayed in water agar on *Glomerella cingulata*

Lignin-like preparation and extraction solvent[a]	Lowest concentration giving maximum inhibition, mg/ml	Germ tube length, μ	% germination
Wheat straw			
Dioxane	0.1	40[b]	60
Ethanol	0.2	120[b]	80
Sulfuric acid	3.0	400	92
Conover loam			
Dioxane	0.2	100[b]	16
Ethanol	0.2	160[b]	70
Muck			
Dioxane	0.2	90[b]	60
Ethanol	0.2	180[b]	72
Buffered water agar		310	96
2% ethanol in buffered agar		300	92

[a] Ethanol solutions of the preparations were suspended in molten agar containing 0.1 *M* phosphate buffer at pH 7.0.

[b] Germ tubes were swollen and distorted and cell walls were thickened.

Laboratory, University of Michigan, Ann Arbor, using a Perkin-Elmer model 21 spectrograph.

All experiments were repeated at least once.

RESULTS.— *Extracts of soil organic matter.*—Several organic matter fractions of soils were tested for fungitoxicity in water agar. α and β humus extracted from loam and muck soils (22) and a highly purified humic acid (10) were assayed at final concentrations of 1-10 mg/ml. Lipids extracted with petroleum ether at 24°C were tested at final concentrations of 0.1-1.0 mg/ml. These preparations were not toxic to germination of conidia or to germ tubes of *G. cingulata*, *F. oxysporum* f. *lycopersici*, or *P. frequentans*. Other work had shown that water extracts and organic solvent extracts of soils also were noninhibitory to spores of these fungi (13). On the contrary, all of these preparations always stimulated growth of germ tubes and mycelium. When washed, defatted muck soil or wheat straw was extracted with ethanol or dioxane (1), however, a fungitoxic fraction was obtained (Table 1). These preparations yielded up to 0.8, 0.3, and 1.2 g/100 g of dry material, respectively; and were assayed in 2% water agar at concentrations of 0.1-3.0 mg/ml. Some preparations gave almost complete inhibition of germination, but more frequently 60-80% of the conidia germinated. Where germination occurred, toxicity was expressed by inhibition of growth of germ tubes and mycelium; and by swellings and distortions of conidia and hyphae. Fungitoxicity was not increased above concentrations of approximately 0.1-0.2 mg/ml. Sulfuric acid lignin (1) from wheat straw was nontoxic.

Levels of fungitoxicity of the lignicolous soil and wheat straw preparations reported in the present work are believed valid; preparations obtained using the non-peroxide forming solvents, petroleum ether and ethanol, for defatting and extraction, respectively, were toxic to fungus spores and germ tubes.

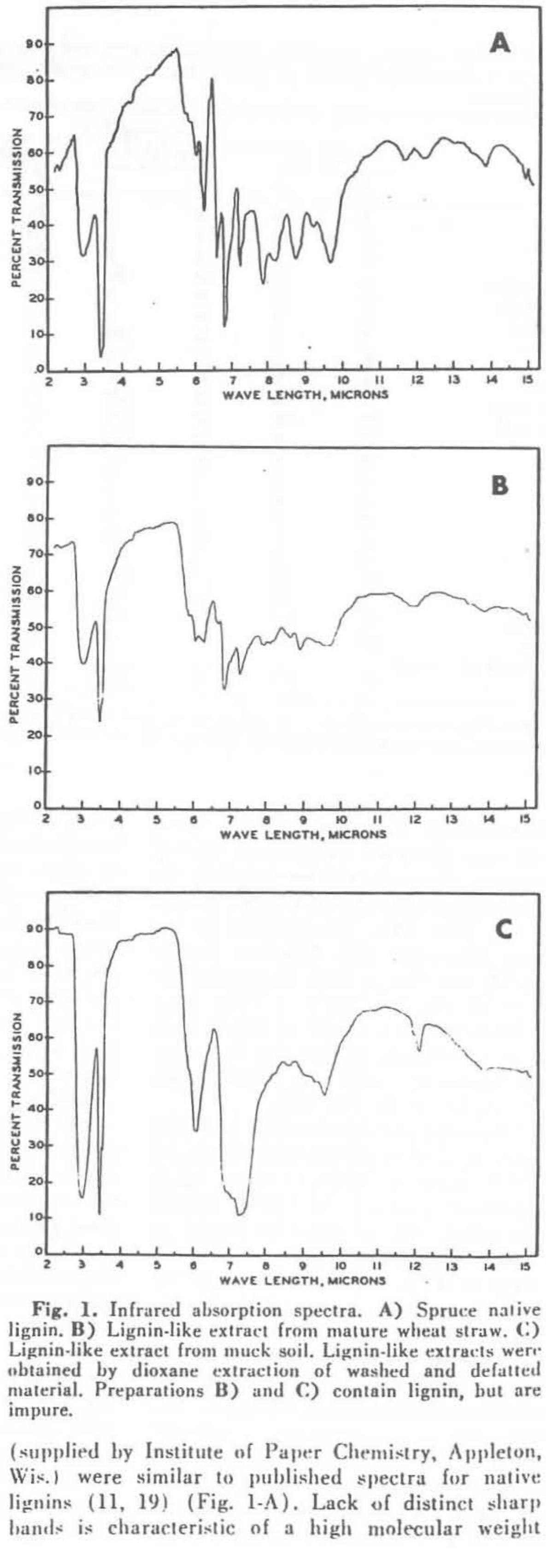

Fig. 1. Infrared absorption spectra. **A)** Spruce native lignin. **B)** Lignin-like extract from mature wheat straw. **C)** Lignin-like extract from muck soil. Lignin-like extracts were obtained by dioxane extraction of washed and defatted material. Preparations **B)** and **C)** contain lignin, but are impure.

Infrared absorption spectra of spruce native lignin (supplied by Institute of Paper Chemistry, Appleton, Wis.) were similar to published spectra for native lignins (11, 19) (Fig. 1-A). Lack of distinct sharp bands is characteristic of a high molecular weight

TABLE 2. Inhibitive effect of lignin monomers, model substances, and decomposition products at various molar concentrations on germination of conidia and growth of germ tubes of *Glomerella cingulata*, *Fusarium oxysporum* f. *lycopersici*, and *Penicillium frequentans*[a]

	G. cingulata						*F. oxysporum*		*P. frequentans*	
	10^{-2} *M*		3×10^{-3} *M*		10^{-3} *M*		3×10^{-3} *M*		3×10^{-3} *M*	
Test compound	%	μ	%	μ	%	μ	%	μ	%	μ
Cinnamyl alcohol	0	0	0	0	16	60	0	0	0	0
Cinnamaldehyde	0	0	0	0	0	0	0	0	0	0
Cinnamic acid	0	0	0	0	96	300	0	0	0	0
Hydrocinnamyl alcohol	0	0	67	20	80	55	60	5	0	0
Hydrocinnamaldehyde	0	0	0	0	74	20	0	0	0	0
Hydrocinnamic acid	0	0	0	0	95	250	0	0	0	0
Phenyl-2-propanone	0	0	90	100	90	120	93	50	94	50
Phenyl acetate	0	0	76	12	90	200	80	10	0	0
Phenyl propionate	0	0	90	60	92	300	82	30	0	0
p-methylcinnamic acid	0	0	92	120	95	120	88	45	95	98
p-hydroxybenzoic acid	15	100	60	150	60	150	98	300	96	400
o-methoxyphenyl	0	0	72	120	95	120	93	250	90	60
Guaiacol	0	0	90	65	90	60	90	90	3	25
Vanillin	0	0	6	10	85	45	0	0	1	5
Ethyl vanillate	0	0	0	0	10	40	0	0	0	0
Eugenol	0	0	0	0	0	0	0	0	0	0
Ferulic acid	0	0	99	120	95	120	90	200	92	210
Veratraldehyde	0	0	9	40	90	300	59	3	0	0
Syringaldehyde	11	30	92	180	90	300	96	30	95	120
Syringic acid	15	60	90	200	92	300	98	200	98	400
Spruce native lignin	90	410	95	300	92	300	95	200	95	200
Buffered water agar	90	400	96	320	95	300	96	265	93	250
3% ethanol in buffered water agar	90	390	92	300	90	280	93	250	92	200

[a] Ethanol solutions of the compounds were mixed in water agar containing 0.1 *M* phosphate buffer at pH 6.4. Results were taken after 24 hours and were based on counts of 300 spores. % = mean percentage of germination; μ = mean length of germ tubes.

amorphous substance (11). The spectra for the wheat straw (extracted with dioxane) preparation showed absorption maxima at the positions characteristic for native lignin, specifically at 2.9, 5.8, 5.9, 6.2, 7.0, 7.9, 8.1, and 12.0 μ (11, 19) (Fig. 1-B). The spectrum for the muck preparation (extracted with dioxane) showed bands at 2.9, 5.9, 7.0, and 12.0 μ; weak or questionable bands occurred at 5.8, 6.6, 7.9, and 8.1 μ (Fig. 1-C). These data and differences in intensity of bands, compared with those of native lignin, suggest that the preparations contained lignin; but were very impure (personal communication, Dr. H. M. Randall).

Extracts of soil amended with lignin.—The inhibitive effects of the impure lignin-like preparations suggested that the source of fungitoxicity might be either lignin itself or decomposition products of lignin. Spruce native lignin was mixed with 12 times its weight of Conover loam soil in petri dishes, moistened, and incubated for 7 days at 24°C. Samples then were extracted with 95% ethanol until no more residue was obtained by evaporating a drop on a microscope slide. The extracts were pooled, evaporated to dryness, redissolved in ethanol, and assayed in water agar at concentrations of 0.1-2.0 mg/ml. In 3 tests, extracts of soil amended with native lignin permitted only 3-70% germination of conidia of *G. cingulata*, compared with 95% germination with native lignin, extracts of unamended soil, or water agar alone. Maximum inhibition was obtained at a concentration of 0.6 mg/ml. When germination occurred, germ tubes were much inhibited by ethanol extracts of lignin-amended soil. Length of germ tubes after 24 hours was 15-85 μ, whereas spruce native lignin and ethanol extracts from unamended soil were stimulatory to fungi (410-450 μ). Water agar gave germ tube lengths of 300-320 μ. These results suggested that lignin had been partially decomposed by soil microorganisms to give fungitoxic products; and that fungitoxicity of lignin-like extracts from soil and wheat straw was due to compounds other than lignin, possibly to decomposition products of lignin.

Inhibitive properties of compounds related to lignin.—The inhibitive properties of the impure lignin-like preparations from straw and soils and of extracts of soils amended with lignin suggested that lignin monomers and model substances including known decomposition products of lignin should be tested for toxicity to fungi. The compounds used were: cinnamyl alcohol, cinnamaldehyde, cinnamic acid, hydrocinnamyl alcohol, hydrocinnamaldehyde, hydrocinnamic acid, phenyl-2-propanone, phenyl acetate, phenyl propionate, *p*-methylcinnamic acid, *p*-hydroxybenzoic acid, *o*-methoxyphenyl acetate, guaiacol, vanillin, ethyl vanillate, eugenol, ferulic acid, veratraldehyde, syringaldehyde, syringic acid, spruce native lignin. Ethyl vanillate was supplied by the Institute of Paper Chemistry, Appleton, Wis.; *p*-methylcinnamic acid was specially prepared for our use by Aldrich Chemical Co., Milwaukee, Wis. Solutions of these compounds in 95% ethanol were mixed with agar to make final dilutions

TABLE 3. Effect of pH of medium and autoclaving on fungitoxicity of spruce native lignin, lignin monomers, and model substances in water agar as determined by length of germ tubes of *Glomerella cingulata*

	Relative toxicity at 3 pH levels	
Chemical ($3 \times 10^{-3} M$)	Not autoclaved	Autoclaved
Cinnamyl alcohol	No germination	No germination
Cinnamaldehyde	do	do
Cinnamic acid	$5.5 = 6.4 > 8.5$	$6.4 > 5.5 > 8.5$
Hydrocinnamyl alcohol	$6.4 > 8.5 > 5.5$	$6.4 > 5.5 > 8.5$
Hydrocinnamaldehyde	$5.5 > 6.4 = 8.5$	No germination
Hydrocinnamic acid	$5.5 = 6.4 > 8.5$	$5.5 = 6.4 > 8.5$
Phenyl-2-propanone	$6.4 > 5.5 > 8.5$	$8.5 > 5.5 > 6.4$
Phenyl acetate	$6.4 > 8.5 > 5.5$	$5.5 > 6.4 = 8.5$
Phenyl propionate	$6.4 > 8.5 > 5.5$	$5.5 > 6.4 = 8.5$
p-methylcinnamic acid	$5.5 = 6.4 > 8.5$	$6.4 > 5.5 > 8.5$
p-hydroxybenzoic acid	$5.5 > 6.4 = 8.5$	$6.4 > 5.5 > 8.5$
o-methoxyphenyl acetate	$5.5 > 6.4 = 8.5$	No inhibition
Guaiacol	$6.4 > 5.5 > 8.5$	$6.4 > 5.5 > 8.5$
Vanillin	$6.4 > 5.5 > 8.5$	$6.4 > 8.5 > 5.5$
Ethyl vanillate	No germination	No germination
Eugenol	do	do
Ferulic acid	No inhibition	No inhibition
Veratraldehyde	$6.4 > 8.5 > 5.5$	No germination
Syringaldehyde	$6.4 > 5.5 > 8.5$	$5.5 = 6.4 > 8.5$
Syringic acid	$5.5 > 6.4 = 8.5$	$5.5 > 6.4 = 8.5$
Spruce native lignin	$5.5 > 6.4 = 8.5$	$5.5 > 6.4 = 8.5$

of $10^{-2} M$, $3 \times 10^{-3} M$, and $10^{-3} M$. Molecular weight of spruce native lignin was taken as 840 (1).

All of the chemicals used, except spruce native lignin, prevented germination of 85% or more of conidia of *G. cingulata* (Table 2) at the $10^{-2} M$ concentration (1.3-1.9 mg/ml). Cinnamyl alcohol, cinnamaldehyde, cinnamic acid, hydrocinnamaldehyde, hydrocinnamic acid, vanillin, ethyl vanillate, eugenol, and veratraldehyde were strongly inhibitory to spore germination at the $3 \times 10^{-3} M$ concentration. Cinnamyl alcohol, cinnamaldehyde, ethyl vanillate, and eugenol were strongly inhibitory at $10^{-3} M$ (130-190 μg/ml). Results were qualitatively similar when *F. oxysporum* f. *lycopersici* and *P. frequentans* were used as test fungi. The response of each of the 3 fungi to the test compounds at concentrations of $3 \times 10^{-3} M$ is in Table 2. *G. cingulata* and *F. oxysporum* f. *lycopersici* responded similarly, whereas *P. frequentans* was somewhat more sensitive. Germination of conidia of this fungus was strongly inhibited by hydrocinnamyl alcohol, phenyl acetate, phenyl propionate, and guaiacol in addition to the compounds listed above. Even at levels permitting germination, there was in almost every case strong inhibition of growth of germ tubes of all 3 test fungi. Further, spores were often markedly enlarged whether germinated or not. It is of interest that *p*-hydroxybenzoic acid, vanillin, and syringaldehyde, 3 well-known oxidation products of lignin, were among the compounds tested.

Since lignin readily undergoes oxidation (1) and this process is influenced by pH and temperature, the effect of pH of the medium and of autoclaving on toxicity of test compounds was studied. Water agar containing the compounds at $3 \times 10^{-3} M$, a concentration near the mean minimum inhibitory level for the group of compounds, was buffered with 0.1 *M* phosphate buffer at pH 5.5, 6.4, or 8.5. Autoclaving altered pH no more than ± 0.1 unit. All 3 test fungi germinated normally at these pH levels. Results based on inhibition of germ tube growth for *G. cingulata* are in Table 3. Autoclaving for 30 minutes at 15 psi increased the toxicity of some compounds and decreased that of others. In some cases, the effect of autoclaving depended on the pH. For example, toxicity of some compounds was decreased by autoclaving at pH 5.5; but was increased by autoclaving at pH 8.5, or *vice-versa*. Relative toxicity at the 3 pH levels depended on the compound and autoclaving. Spruce native lignin markedly inhibited growth of germ tubes at pH 5.5, but not at higher pH levels. There was no consistent relationship of toxicity to either pH or autoclaving. The changes in toxicity with pH and autoclaving probably reflect formation of derivatives due to oxidation or other reactions.

Soil fungistasis as assayed by agar discs can be annulled by adding dilute peptone solution to the agar (13). Therefore, 0.2 ml of 0.5% peptone was applied to 1.5×7.5 mm agar discs containing conidia and certain of the inhibitory compounds at concentrations of $3 \times 10^{-3} M$ or $10^{-2} M$. Spores of 1 or more of the 3 test fungi were stimulated to germinate when previously inhibited by cinnamic acid, *p*-methylcinnamic acid, *o*-methoxyphenyl acetate, guaiacol, vanillin, ferulic acid, or veratraldehyde. Toxicity by cinnamyl alcohol, cinnameldehyde, hydrocinnamyl alcohol, hydrocinnamaldehyde, hydrocinnamic acid, and eugenol was not reversed. Possibly, inhibition would have been annulled if a minimal inhibitory concentration of the individual chemical had been ascertained and tested.

DISCUSSION.—The identity of the fungitoxic material in the lignin-like extracts is not known, but lignin decay products could have been extracted with the solvents used. Infrared absorption spectra for these preparations indicated that materials other than lignin were present. Known oxidation products of lignin include products, could have been extracted with the solvents *p*-hydroxybenzaldehyde, guaiacol, protocatechuic acid, veratric acid, vanillin, syringaldehyde, veratric aldehyde, and others (1). Oxidative breakdown products of lignin such as vanillic and syringic acids, were released by microbial decay from birch and spruce sawdusts and were presumed to occur as a result of lignin decomposition (7); but possible synthesis from carbohydrates or their presence as such in wood (1, 15) were not ruled out. In this connection, vanillin has been isolated from soil (17) and was considered present as such in these samples. Lignin decomposition products and other compounds related to lignin building units were fungitoxic in the work reported here. Fungitoxicity of some of the compounds tested, including vanillin (3, 16), eugenol, and cinnamaldehyde (14), is already known.

Under aerobic microbial degradation, lignin would be expected to yield phenolic compounds toxic to fungi in the vicinity of decaying lignin particles; and hence might play a significant role in the reported beneficial effects of plant residues, particularly of mature plants, on soil-borne fungus diseases (2, 18). To our knowledge, this possibility has not heretofore been suggested. It is supported by the demonstrated fungitoxicity of the impure lignin-like preparations obtained with mild reagents from soil and wheat straw and by the fungitoxicity of lignin monomers and model substances including known decomposition products of lignin. Some of these were toxic in agar media at 130-190 μg/ml, a concentration which might be attained in soil if a mixture of compounds is considered; and in view of the abundance of lignin in mature vegetation and its known accumulation in soil. Further, these compounds showed increased or decreased toxic effects depending on pH and autoclaving, suggesting that in a mixture, toxicity would be expressed at various soil conditions. The biological formation of fungitoxic substances from lignin in soil also is suggested by our results with extracts from soil to which spruce native lignin had been added.

A possible relation between lignin decomposition products and the widespread soil fungistasis also is suggested by these results, but the fact that soil fungistasis occurs as well in soils low in organic matter as in soils containing substantial organic matter (13) would cast some doubt on lignin decomposition products as a general explanation of soil fungistasis. Nevertheless, the desirability of quantitative determination of chemical compounds related to lignin building units in decaying crop residues and soil organic matter and of estimations of their fungitoxicity is clearly indicated.

LITERATURE CITED

1. Brauns, F. E. 1952. The chemistry of lignin. Academic Press, Inc., New York. 808 p.
2. Davey, C. B., and G. C. Papavizas. 1959. Effect of organic soil amendments on the Rhizoctonia disease of snap beans. Agron. J. 51:493-496.
3. Dittmer, Dorothy S. ed. 1959. Handbook of toxicology. V. Fungicides. W. B. Saunders Co., Philadelphia. 242 p.
4. Flaig, W. 1958. Die Chemie organischer Stoffe in Boden and deren physiologische Wirkung. Verhandlungen der II. und IV. Kommission der Internationalen Bodenkundlichen Gesellschaft Hamburg 1958. Vol. II. p. 11-45.
5. Gottlieb, S., and M. J. Pelczar, jr. 1951. Microbiological aspects of lignin degradation. Bacteriol. Rev. 15:55-76.
6. Gottlieb, S., W. C. Day, and M. J. Pelczar, jr. 1950. The biological degradation of lignin. II. The adaptation of white-rot fungi to growth on lignin media. Phytopathology 40:926-935.
7. Henderson, Moira E. K. 1955. Release of aromatic compounds from birch and spruce sawdusts during decomposition by white-rot fungi. Nature 175:634-635.
8. Henderson, Moira E. K. 1956. A study of the metabolism of phenolic compounds by soil fungi using spore suspensions. J. Gen. Microbiol. 14:684-691.
9. Henderson, Moira E. K., and V. C. Farmer. 1955. Utilization by soil fungi of p-hydroxybenzaldehyde, ferulic acid, syringaldehyde and vanillin. J. Gen. Microbiol. 12:37-46.
10. Jones, U. S. 1947. Availability of humate potassium. Soil Sci. Soc. Am. Proc. 12:373-378.
11. Kudzin, S. F., R. M. DeBaun, and F. F. Nord. 1951. Investigations on lignin and lignification. VI. The comparative evaluation of native lignins. J. Am. Chem. Soc. 73:4615-4618.
12. Lincappa, B. T., and J. L. Lockwood. 1960. Fungistatic effects of lignin, lignin monomers, and model substances. (Abstr.) Phytopathology 50:644.
13. Lincappa, B. T., and J. L. Lockwood. 1962. The nature of the widespread soil fungistasis. J. Gen. Microbiol. 26:473-485.
14. Maruzzella, J. C., D. A. Scrandis, J. B. Scrandis, and G. Grabon. 1960. Action of odoriferous organic chemicals and essential oils on wood-destroying fungi. Plant Disease Reptr. 44:789-792.
15. Roelofsen, P. A. 1959. The plant cell wall. Gebrüder Borntraeger, Berlin-Nikolassee. 335 p.
16. Schaall, L. A., and G. Johnson. 1955. The inhibitory effect of phenolic compounds on the growth of Streptomyces scabies as related to the mechanism of scab resistance. Phytopathology 45:626-628.
17. Shorey, E. C. 1914. The presence of some benzene derivatives in soils. J. Agr. Research 1:357-363.
18. Snyder, W. C., M. N. Schroth, and R. Christou. 1959. Effect of plant residues on root rot of bean. Phytopathology 49:755-756.
19. Stevens, G. de, and F. F. Nord. 1951. Investigations on lignin and lignification. VIII. Isolation and characterization of bagasse native lignin. J. Am. Chem. Soc. 73:4622-4625.
20. Tenney, Florence G., and S. A. Waksman. 1929. Composition of natural organic materials and their decomposition in the soil. IV. The nature and rapidity of decomposition of the various organic complexes in different plant materials, under aerobic conditions. Soil Sci. 28:55-84.
21. Waksman, S. A. 1932. Principles of soil microbiology. 2nd ed. Williams and Wilkins Co., Baltimore. 894 p.
22. Waksman, S. A. 1938. Humus: origin, chemical composition, and importance in nature. Williams and Wilkins Co., Baltimore. 526 p.
23. Waksman, S. A., and K. R. N. Iyer. 1932. Contributions to our knowledge of the chemical nature and origin of humus. I. On the synthesis of the "humus nucleus." Soil Sci. 34:43-69.